

Distribution analysis of ultra-high molecular mass poly(ethylene oxide) containing silica particles by size-exclusion chromatography with dual light-scattering and refractometric detection

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Abstract

Two different size-exclusion chromatography (SEC) systems, connected in-line either to a low-angle light scattering (LALS) or to a multi-angle light scattering (MALS) detector, are employed for determination of molecular mass distributions (MMD) of poly(ethylene oxide) (PEO) samples having a weight average molecular mass up to eight millions. The detrimental effect of the presence of strongly scattering silica particles in the samples on the light scattering signal can be eliminated using a suitable sample dissolution procedure utilizing silica solubility in aqueous mobile phase. The selection of flow-rate and sample concentration have a large impact on the obtained results. Hydrodynamic retardation phenomena and nonlinearity effects are shown to introduce severe errors in the molecular mass distributions unless flow-rate and sample concentration are kept at sufficiently low levels. Self-compensating ability of the dual detection in flow-rate effects is shown to be the main advantage here. A good agreement between the results obtained using LALS and MALS detection is found provided that a carefully selected angular extrapolation procedure is used in the case of MALS data. Thus, using carefully selected experimental conditions, SEC with light-scattering (LS) and refractometric detection proved to be an efficient technique for MMD characterisation also of ultra-high molecular mass (UHM) PEO polymers.

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1. Introduction

Ultra-high molecular mass poly(ethylene oxide) (PEO) is widely used in various industrial applications as thickeners, flocculants and flow-improving agents [1]. Commercial products, which are not expected to have narrow molecular mass distributions (MMD), are usually characterized only by approximate molecular mass and solution viscosity at a fixed concentration. Although this may be adequate in large-scale uses, such as concrete pumping [1], more precise characterization of their specific pharmaceutical qualities in terms of molecular mass and its distribution is highly desirable.

Size-exclusion chromatography of ultra-high molecular mass (UHM) water-soluble polymers having broad molecular mass distributions is still a challenge. General obstacles to be expected here were summarized by Giddings [2]: (1) flow suppression in pores, (2) shear degradation, (3) non-equilibrium transport between mobile and stationary phase, (4) group of hydrodynamic phenomena termed as polarization effect, hydrodynamically induced diffusion, stress induced diffusion, and multipath effect, all of them leading to retardation of large coils, (5) hydrodynamic chromatography mode leading to accelerated elution, (6) concentration dependent partition coefficient (non-linearity).

Flow suppression in pores is necessary to maintain SEC resolution. Separation of UHM polymers requires sufficiently wide pores that must have sufficient depth to keep stag-

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nant mobile phase. It is easy to calculate that PEO sample having $M_w = 10^6$ and $M_w/M_n = 3$ and, if described by Pearson's distribution, would contain molecules in the M range from 10^5 to 2×10^7 . For the same polymer, described by Schulz–Zimm distribution, would follow the M range from 10^3 to 10^7 . From PEO data of Devanand and Selzer [3], gyration radius $r_g = 388$ nm can be calculated for $M = 2 \times 10^7$ and transformed to hydrodynamic diameter ca. 600 nm using [4] $r_g/r_h = 1.33$, where r_h is hydrodynamic radius. Giddings's calculations [2] then predict a minimum column packing particle size above $15 \mu\text{m}$ to satisfy the condition of pore flow suppression for a broad PEO having not exceptionally high $M_w = 10^6$. This should be borne in mind any time when broad UHM polymers are analyzed by a SEC technique.

UHM polymers have to be expected to be particularly susceptible to shear degradation during SEC analysis. There is a common agreement that shear force, being a product of velocity gradient and solvent viscosity, is a decisive parameter here. Thus, water-soluble polymers should be more prone to shear degradation, when compared with organic polymers of the same M in tetrahydrofuran (THF), by a factor of two resulting from solvent viscosity difference. A natural requirement for lowering flow-rate to reduce velocity gradient contradicts high-speed SEC experiments here. Shear forces generated in the packed bed are assumed to be the main source of shear degradation. Barth [5] pointed out that extra-column sources of degradation (injection valve, capillary tubing and column frits) cannot be neglected because quite high shear forces can be generated especially in modern hardware optimized to minimize extra-column band broadening. Numerous studies of polymer degradation during SEC analysis have been published, mostly using narrow MMD polystyrenes in THF [6–11]. Sometimes contradictory results may be probably ascribed to the differences in extra-column hardware used by various authors. The recommended conditions [11] to avoid degradation of polystyrene ($M = 17 \times 10^6$) in THF were flow-rate ≤ 0.2 ml/min, column particle size $20 \mu\text{m}$ and injected concentration $\leq 0.01\%$. There is rather general agreement that below a certain critical molecular mass no chain rupture takes place at a given shear stress. This implies further difficulties in the case of polymers having broad MMD [2,12]; also, great differences in sensitivity to shear stress may be observed for different polymers, as shown by easily degradable PEO and non degradable carboxymethyl cellulose under identical conditions [13].

Non-equilibrium in the stationary phase, which is controlled by the diffusion into and out of the pores, becomes increasingly important for UHM polymers. The relevant effective diffusion coefficient is proportional to the bulk solute-solvent diffusion coefficient multiplied by an obstructive factor characterizing pore network of support particles [2]. Because bulk diffusion coefficient depends on $M^{-\alpha}$, where α is usually between 0.5 and 0.6, this effect must be also expected to vary along the MMD distribution in the case of broad UHM polymer.

Briefly speaking, retardation of UHM polymers summarized as a group of hydrodynamic phenomena [2] results from a complex flow pattern due to hydrodynamic, diffusion and inertial forces acting on very large coils in SEC columns. Several authors noticed these effects [2,5,7,9,14]; the dominant mechanism is difficult to distinguish because all of them yield particle retardation.

The hydrodynamic chromatography mode has been shown to give similar elution order to that of SEC (the largest coils eluted first) on nonporous packed beds [15,16]. This may be relevant in the case of UHM polymers as a transition region close to and above the exclusion limit of SEC columns [17,18].

Non-linearity, which manifests itself as a concentration-dependent elution volume, is related to changes of polymer hydrodynamic volume as a decisive separation parameter. Because polymer concentration varies within a polymer peak as well as along the SEC column due to successive band broadening, this effect, if present, would affect the shape of the eluted peak in a complex way. The general assumption in SEC that concentration (variable from the baseline to the peak top) is low enough to allow the use of infinite dilution approximation may not be true in the case of UHM polymers. Model calculations of concentration-dependent elution volumes [19,20] indicate a clear connection to the changes of polymer coil dimensions with concentration as manifested by concentration dependence of reduced viscosity (Huggins equation) or reduction of intensity of scattered light (second virial coefficient term). The coil overlap concentration $c^* \approx 1/[\eta]$, where $[\eta]$ is intrinsic viscosity, means a concentration where coils just touch each other in bulk solvent volume; it is mostly used as a criterion of sufficient dilution. Model Brownian dynamics simulations have shown [21] that a significant chain overlap is observed even at concentrations as low as $0.3c^*$. Hence, the concentration where coil interactions become negligible should be considerably lowered below c^* . For instance, to obtain concentration-independent elution volumes for hyaluronic acid [22], injected polymer concentration had to be reduced below 0.01% for M around 10^6 . The value of $0.3c^*$ for PEO having $M = 10^7$ can be estimated to be around 0.008% using the Mark–Houwink equation for PEO from [1]. Because on-column dilution may be expected to reduce solute concentration by a factor of ten for a broad polymer sample, a very high sensitivity and baseline stability of a differential refractometer (DRI) are required.

To find conditions of a correct SEC analysis of UHM PEO, free of all effects discussed above, seems impossible if the system used is equipped with a DRI unit only, because no narrow standards exist in this range. The addition of a molecular mass-sensitive detector will allow differentiating between possible shear degradation and other flow-rate and molecular mass-dependent detrimental effects. These effects should lead to distortions of the $\log M$ versus elution volume calibration, accessible when a combination of a light-scattering (LS) and DRI detection is used. The use of a SEC column

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